assessments of the antibacterial property of mineral trioxide aggregate and glass ionomer cement mixture

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AKL performed the study and statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AK designed the study and managed the analyses of the study. Author MNP assisted in microbiological evaluation.

All authors read and approved the final manuscript.

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ABSTRACT

Mineral trioxide aggregate (MTA) is an experimental material used for the capping of pulps as well as a root end filling material. Glass ionomer cement (GIC) is a widely used dental restorative due to its high mechanical strength and antibacterial function. The aim of this study is to assess the antibacterial property of MTA when mixed with GIC. The study comprised four groups with 5 samples each: Group A (MTA), group B (GIC), groups C (MTA+GIC+MTA Liquid) and group D (MTA+GIC+GIC Liquid). All samples were tested against Enterococcus faecalis using Agar well diffusion method in Tryptone soya agar. The One-way ANOVA test was employed using the

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1. INTRODUCTION

Mineral trioxide aggregate (MTA) cement is composed of Portland cement mixed with bismuth sulfate. The main constituent phases are tricalcium and dicalcium silicate and tricalcium aluminate [1]. It has various uses in endodontics [2]. Its potential is exhibited mainly as a pulp-capping material during vital pulp therapy [3]. It has various other uses including capping of pulps with reversible pulpitis, apexification, repair of root perforations as well as a root end filling material [4]. It favours the occurrence of apexification and periapical healing after root canal preparation [5]. MTA has shown effectiveness in resolving periodontitis [6]. MTA has excellent biocompatibility, but it is often not preferred as being an expensive material [7]. MTA is an ideal material for many endodontic procedures because it allows the growth of cementum and periodontal ligament leading to effective periodontal healing [8]. It has been stated that MTA does not have good antibacterial action against E. faecalis after setting as it does before setting [9]. However, MTA when mixed with chlorhexidine instead of sterile water showed increased cytotoxicity of the resulting MTA-based material formed [10].

Glass Ionomer Cement (GIC) is the product of the reaction between ion leachable glass or silica and an aqueous solution of polyacrylic acid [11]. GIC is preferred because of its fluoride releasing property, use as atraumatic restorative material and because it is tooth coloured [12]. It is an ideal dentine substitute because of its anticariogenic property, stable ionic bonding, high mechanical strength and antibacterial function [13,14]. It has been preferred because of its ability to exhibit antibacterial property for a long period of time due to its fluoride releasing capacity [15]. MTA when mixed with GIC has shown good biocompatibility [16]. Also the setting time of MTA when mixed with GIC is improved compared to MTA alone [17]. Thus we have chosen to use this mixture for our study.

The antibacterial property of this mixture was studied against E. faecalis. E. faecalis is the most common organism found in failed endodontically treated teeth [18]. This is due to its capability to invade dentinal tubules [19]. E. faecalis has shown resistance to antibiotics such as erythromycin and azithromycin [20]. Its ability to compete with other microorganisms, invade dentinal tubules and resist nutritional deprivation has been stated as reasons for its persistence in endodontic diseases [21].

The aim of this study is to assess the antibacterial property of Mineral trioxide aggregate when mixed with Glass ionomer cement.

2. MATERIALS AND METHODS

The study comprised 4 groups with 5 samples each: Group A (MTA), Group B (GIC), Group C (MTA+GIC+MTA Liquid) and Group D (MTA+GIC+GIC Liquid). Vishal Apiseal® MTA was used for the study. The MTA and GIC powders were mixed in a ratio of 1:1. Agar well diffusion method was used to study the antibacterial efficacy of the study groups. The study was conducted on Trypthon soya agar which was poured in 5 sterilised petri plates. The plates were streaked with strains of Enterococcus faecalis. The trypthon soya agar plates and standard strain of the bacteria maintained in the Department of Microbiology, Saveetha Dental College and Hospital was used for the study. 4 uniform wells (4 mm diameter each) were punched at equidistant points in the agar plate by use of a sterile copper coil. The wells were filled immediately by the 4 study group samples after being mixed according to the manufacturer's instructions. The plates were then incubated at 37°C for 24 hours. The zone of inhibition was measured with a millimeter scale with an accuracy of 0.5 mm in two perpendicular locations for each study group by an independent observer (Fig. 2).
2.1 Statistical Analysis

Data was recorded in Microsoft Excel and then subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) for MacOS (Version 19, 2010). One-way ANOVA test was employed with the level of significance set at $P<0.05$. Post Hoc test namely Tukey HSD was done and multiple comparisons between the study groups were obtained. Appropriate graphs, tables and charts were constructed using the same software for clear representation of the results obtained.

3. RESULTS AND DISCUSSION

The average zone of inhibition of group A was $18.6 \pm 1.714 \text{ mm}$, group B was $0.4 \pm 0.548 \text{ mm}$, group C was $18.6 \pm 2.608 \text{ mm}$ and group D was $12.8 \pm 2.168 \text{ mm}$ (Fig. 1, Table 1). Overall there was a statistically significant difference between the zones of inhibition of the 4 study groups, $F(3,16)=11.631, P<0.001$ (Table 2). Post Hoc tests revealed that the mean difference between the zones of inhibition of Group A and Group B was $18.200$ (statistically significant, $P=0.001$), between Group A and Group C was $0.000$ (statistically not significant, $P=1.000$), between Group A and Group D was $5.800$ (statistically not significant, $P=0.391$), between Group B and Group C was $18.200$ (statistically significant, $P=0.001$), between Group B and Group D was $12.400$ (statistically significant, $P=0.015$) and between Group C and Group D was $5.800$ (statistically not significant, $P=0.391$) (Table 3).

![Average Zone of Inhibition (mm)](image)

**Fig. 1.** Bar graph showing average zone of inhibition (in mm) of the 4 study groups

Bar graph showing average zone of inhibition (in mm) of the 4 study groups namely, MTA (blue-group A), GIC (black-group B), MTA+GIC+MTA Liquid (green-group C) and MTA+GIC+GIC Liquid (yellow-group D), against *E. faecalis* after inoculation for 24 hours in Tryptone soya agar. From the graph it is inferred that MTA group and MTA+GIC+MTA Liquid groups exhibited higher zones of inhibition against *E. faecalis*.

**Table 1.** Table showing descriptive statistics of the mean zones of inhibition of the 4 study groups

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA</td>
<td>5</td>
<td>18.60</td>
<td>1.714</td>
<td>4.792</td>
<td>5.30</td>
<td>31.90</td>
<td></td>
</tr>
<tr>
<td>GIC</td>
<td>5</td>
<td>0.40</td>
<td>0.548</td>
<td>0.245</td>
<td>-0.28</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>MTA+GIC+MTA Liquid</td>
<td>5</td>
<td>18.60</td>
<td>2.608</td>
<td>1.166</td>
<td>15.36</td>
<td>21.84</td>
<td></td>
</tr>
<tr>
<td>MTA+GIC+GIC Liquid</td>
<td>5</td>
<td>12.80</td>
<td>2.168</td>
<td>0.970</td>
<td>10.11</td>
<td>15.49</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>12.60</td>
<td>9.208</td>
<td>2.059</td>
<td>8.29</td>
<td>16.91</td>
<td></td>
</tr>
</tbody>
</table>

Namely, MTA (Group A), GIC (Group B), MTA+GIC+MTA Liquid (Group C) and MTA+GIC+GIC Liquid (Group D), against *E. faecalis*. From the table it is evident that Groups A and B showed the highest mean zones of inhibition.
Fig. 2. Image showing Zone of inhibition of the 4 study groups in one sample

Image showing zone of inhibition (in mm) of the 4 study groups namely, MTA (group A), GIC (group B), MTA+GIC+MTA Liquid (group C) and MTA+GIC+GIC Liquid (group D), against E. faecalis after inoculation for 24 hours in Tryptone soya agar in one sample.

Table 2. Table showing results from the One-way ANOVA test between the 4 study groups

<table>
<thead>
<tr>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1104.400</td>
<td>3</td>
<td>368.133</td>
<td>11.631</td>
</tr>
<tr>
<td>Within Groups</td>
<td>506.400</td>
<td>16</td>
<td>31.650</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1610.800</td>
<td>19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Namely, MTA (Group A), GIC (Group B), MTA+GIC+MTA Liquid (Group C) and MTA+GIC+GIC Liquid (Group D).

(Data is statistically significant; P=0.000; P<0.05)

Table 3. Table representing the results from the Post Hoc test done between the 4 study groups

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean difference (I-J)</th>
<th>Std. error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA</td>
<td>GIC</td>
<td>18.200</td>
<td>3.558</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MTA+GIC+MTA Liquid</td>
<td>0.000</td>
<td>3.558</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>MTA+GIC+GIC Liquid</td>
<td>5.800</td>
<td>3.558</td>
<td>0.391</td>
</tr>
<tr>
<td>GIC</td>
<td>MTA</td>
<td>-18.200</td>
<td>3.558</td>
<td>0.001</td>
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<td>MTA+GIC+MTA Liquid</td>
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<td>1.000</td>
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<tr>
<td></td>
<td>GIC</td>
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<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MTA+GIC+MTA Liquid</td>
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<td>MTA+GIC+MTA Liquid</td>
<td>-5.800</td>
<td>3.558</td>
<td>0.391</td>
</tr>
</tbody>
</table>

It revealed that the mean difference between the zones of inhibition of Group A (MTA) and Group B (GIC) was statistically significant (P=0.001), between Group A (MTA) and Group C (MTA+GIC+MTA Liquid) was statistically not significant (P=1.000), between Group A (MTA) and Group D (MTA+GIC+GIC Liquid) was statistically not significant (P=0.391), between Group B (GIC) and Group C (MTA+GIC+MTA Liquid) was statistically significant (P=0.001), between Group B (GIC) and Group D (MTA+GIC+GIC Liquid) was statistically significant (P=0.015) and between Group C (MTA+GIC+MTA Liquid) and Group D (MTA+GIC+GIC Liquid) was statistically not significant (P=0.391).
In the present study it was observed that MTA, and MTA when mixed with GIC + MTA liquid had the same inhibitory effect on *Enterococcus faecalis* sp. with the average zone of inhibition being 18.6 ± 1.714 mm and 18.6 ± 2.608 mm respectively. GIC alone had very poor antibacterial activity on the *E. faecalis* sp. with the average zone of inhibition being 0.4 ± 0.548 mm. When MTA powder is mixed with GIC and GIC liquid the zone of inhibition averages to 12.8 ± 2.168 mm.

In this study, the bacterial inhibitory effect is studied against *Enterococcus faecalis* as it is often found in cases of failed endodontic therapy [22].

According to a study by Sipert et al. 2005, MTA showed antibacterial activity against *E. faecalis*, *Micrococcus luteus*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [23]. In a study by Huang et al. 2019, MTA had high antibacterial activity and even some anti-inflammatory activity [24]. Another study showed that Intermediate Restorative Material (IRM) and MTA had higher antibacterial activity than other root end filling materials such as amalgam, Geristore®, Dyract®, Clearfil APX composite with SE Bond® etc [25]. It has been stated that MTA (both white and gray) has poorer antibacterial efficacy when compared to calcium hydroxide and new endodontic cement (NEC) [7].

In an antimicrobial test done against 4 bacterial strains, MTA failed to inhibit *E. faecalis* and *E. coli* [26]. However in the present study, MTA and the various combinations of MTA with GIC has shown considerable inhibitory capacity against *E. faecalis*. In a test done using MTA from various brands only Endocem MTA® showed inhibitory capacity against *E. faecalis* [27]. To standardise the antibacterial efficacy of MTA, tests need to be done using MTA produced by various brands. A systematic review of the antibacterial efficacy of various root canal sealers against *E. faecalis* stated that the inhibitory effect was observed only within the first 24 hours and diminished over the next 2-3 days. However, no study in this review considered a mixture of MTA with any other material [28]. No study has been done in the past which compares the antibacterial property of MTA when mixed with GIC.

Thus, further study needs to be done to confirm the antibacterial property of MTA by using MTA produced by different manufacturers, checking the zone of inhibition at different time intervals and by mixing it in different proportions with GIC for better understanding. Clinical tests are required for standardisation of the antibacterial property of the mixture. The fracture strength and longevity of the mixture has to be considered before bringing it into clinical practice.

4. CONCLUSION

Within the limits of the study it can be concluded that the antibacterial activity of MTA and MTA+GIC+MTA liquid is similar but better than other study groups although results need to be compared with MTA produced by other companies to standardise the study and come to a generalised conclusion regarding MTA.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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3. Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp-

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